PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P35460WO/PWC	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/GB2004/004907	International filing date *(day/month/year)* 19.11.2004	Priority date *(day/month/year)* 21.11.2003

International Patent Classification (IPC) or national classification and IPC
C07K14/415

Applicant
UWS VENTURES LIMITED et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 1-6 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20.07.2005	19.01.2006

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/004907

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-42 as originally filed

Claims, Numbers

1-42 received on 21.09.2005 with letter of 21.09.2005

Drawings, Sheets

1-10 as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 22-23

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 22-23

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-21
	No:	Claims	24-42
Inventive step (IS)	Yes:	Claims	1-21
	No:	Claims	24-42
Industrial applicability (IA)	Yes:	Claims	1-21, 24-42
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

D1: WO 98/55869 A (SENSOR TECHNOLOGIES, INC; WOLF, DAVID, E) 10 December 1998 (1998-12-10)

D2: WO 00/16099 A (SENSOR TECHNOLOGIES, INC) 23 March 2000 (2000-03-23)

D3: MIN W ET AL: "STABILITY AND DETECTION OF RECOMBINANT PRE-PRO-CONCANAVALIN A AFTER CYTOPLASMIC EXPRESSION IN ESCHERICHIA-COLI" 1992, FEBS LETTERS, VOL. 301, NR. 3, PAGE(S) 315-318 , XP001204961 ISSN: 0014-5793

D4: DINCTURK H BENAN ET AL: "Recombinant pre-pro-Concanavalin A (jack bean) is stable but of low solubility" December 2001 (2001-12), JOURNAL OF BIOSCIENCES (BANGALORE), VOL. 26, NR. 5, PAGE(S) 635-640 , XP009043433 ISSN: 0250-5991

D5: MIN W ET AL: "NON-GLYCOSYLATED RECOMBINANT PRO-CONCANAVALIN A IS ACTIVE WITHOUT POLYPEPTIDE CLEAVAGE" EMBO (EUROPEAN MOLECULAR BIOLOGY ORGANIZATION) JOURNAL, vol. 11, no. 4, 1992, pages 1303-1307, XP001205158 ISSN: 0261-4189

Novelty (Article 33.2 PCT) and inventive step (Article 33.3 PCT)

Having regard to **D1 or D2** the subject-matter of claims 24-42 lacks novelty. Documents **D1 or D2** describe already concanvalin A which is inter alia used for measuring glucose concentrations. The new wording of claim 24 is not suitable to clearly distinguish the claimed matter from the cited prior art. Neither is claim 24 restricted to a lectin ConA produced **in bacteria** nor does the term "**substantially**" exclude the presence of glycogen or other impurities from the scope of the claim. At present it cannot be ruled out that the presence of glycogen in the systems described interferes with the binding of concanavalin A to another ligand. Even if the applicant would render the claimed matter formally novel by incorporating additional technical features of true technical nature into the claims, it is not visible which of these technical features possible represents a surprising/unexpected effect. Thus, at present said matter lacks also the required inventive step.

Based on the reasons given above we also maintain our opinion that with regard to

documents **D5**, which describes already methods for the recombinant production of concanavalin A, the subject-matter of claims 24-26 lacks novelty. Concerning your comments with respect to documents **D4-D5** we note that **precursor forms** of concavalin A are specifically claimed in claims 26 and 34 although they are, according to the applicant, not capable to bind glucose. Thus, the term "precursor form" should be deleted from claim 26. Since the applicant does not claim a protein composition or preparation but a "glucose binding protein", eg. a lectin or Concanavalin A (see claims 24-26), it is not visible to this authority how the simple removal of any "impurities" could render the claimed polypeptide novel over the known prior art. In this context the applicant is also reminded that the matter claimed should be defined by positive technical features but not by the absence of something completely undefined.

The subject-matter of claims 1-21 is considered novel and inventive. However, in contrast to the view of the applicant we maintain our position that the broad and imprecise term "non-plant host" should be replaced by "bacterial host" and the imprecise expression "glucose binding protein" should be replaced by "concanavalin A". In its present form claim 1 represents only a desideratum which does not comply with the requirements of Article 5 and 6 PCT.

The novelty of the claimed glucose binding protein with respect to the cited prior art has been discussed by phone with the representative on 13/01/2006 in order to comply with such a request raised by the representative in his letter dated 21/09/200 (see page 5).

CLAIMS

1. A method of obtaining a recombinant glucose binding protein expressed in non-plant host cells comprising reducing the glycogen content of a lysate of said cells.

2. A method as claimed in claim 1 comprising treating a lysate of said cells with a buffer in which glycogen is soluble, but in which said protein is insoluble.

3. A method as claimed in claim 2 wherein other impurities are also soluble in said buffer.

4. A method as claimed in claim 2 or claim 3 wherein said buffer is a low ionic strength buffer ($I < 0.3$) with a pH between 8.5 and 9.5.

5. A method as claimed in claim 4 wherein said buffer further comprises a metal chelating agent.

6. A method as claimed in claim 5 wherein said metal chelating agent is EDTA.

7. A method as claimed in any one of claims 1 to 5 wherein said buffer further comprises a non-ionic detergent.

8. A method as claimed in claim 7 wherein said non-ionic detergent is Triton X-100.

9. A method as claimed in any one of claims 1 to 8 wherein said buffer comprises 2-(cyclohexylamino)-ethanesulphonic acid.

10. A method as claimed in any one of claims 1 to 8 wherein said buffer comprises borate.

11. A method as claimed in claim 10 wherein said buffer is 20 mM Borax ($Na_2B_4O_7.10H_2O$.)

12. A method as claimed in any one of claims 2 to 11 wherein said pH is between 9.05-9.25.

13. A method as claimed in any one of claims 2 to 12 wherein $I < 0.1$.

14. A method as claimed in any one of claims 1 to 13 further comprising the step of removing any glycogen-Con A complex formed.

15. A method as claimed in any one of claims 1 to 14 wherein said non-plant host is a bacterium.

16. A method as claimed in claim 15 wherein said bacterium is *Escherichia coli.*

17. A method as claimed in claim 15 wherein said *Escherichia coli* cells are incapable of producing glycogen due to defects or mutations in genes for the biosynthesis of glycogen.

18. A method as claimed in any one of claims 1 to 17 wherein said non-plant host cells have been cultured in the absence of an assimilable carbohydrate or carbon source that may be accumulated as glycogen.

19. A method as claimed in claim 18 wherein said non-plant host cells have been cultured in the absence of glucose.

20. A method as claimed in any one of claims 1 to 19 wherein said glucose binding protein is a glucose binding lectin.

21. A method as claimed in claim 20 wherein said lectin is Concanavalin A.

22. The use of a buffer in which glycogen is soluble, but in which a glucose binding protein is insoluble in the purification of a recombinant glucose binding protein expressed by a non-plant host cell.

23. The use as claimed in claim 22 modified by any of the features as claimed in any one of claims 2 – 20.

24. A recombinant glucose binding protein that is substantially free of glycogen, and other impurities.

25. A protein as claimed in claim 24, wherein said protein is a lectin.

26. A protein as claimed in claim 25, wherein said lectin is Concanavalin A, or a precursor form, or a mutant, or a variable valency or low valency form thereof.

27. The use of a recombinant glucose binding protein as claimed in claim 24 in a system where the presence of glycogen would interfere with the binding of said glucose binding protein to another ligand.

28. The use as claimed in claim 27 for measuring glucose concentration.

29. The use as claimed in claim 27 or claim 28 wherein the recombinant protein is expressed from a coding sequence derived from a leguminous plant.

30. The use as claimed in claim 29 wherein said plant is of the genus *Canavalia.*

31. The use as claimed in any one of claims 27 to 30wherein said plant is *Canavalia ensiformis.*

32. The use as claimed in any one of claims 27 to 31wherein said protein is a lectin.

33. The use as claimed in any one of claims 27 to 31 wherein said protein is a Concanavalin-A like lectin.

34. The use as claimed in any one of claims 27 to 31wherein said protein is Concanavalin A, or a precursor form, or a mutant, or a variable valency or low valency form thereof, which is substantially free of Con-A-sequence related polypeptides or fragments.

35. The use as claimed in claim 33 wherein said Concanavalin A is in the mature tetrameric tetravalent form.

36. The use as claimed in any one of claims 28 to 35 wherein the protein is substantially free of glycogen.

37. The use as claimed in any one of claims 28 to 36 wherein said glucose concentration is measured by viscometric methods.

38. The use as claimed in any one of claims 28 to 36 wherein said glucose concentration is measured using a fluorescence-based method.

39. The use as claimed in any one of claims 28 to 38 wherein the method utilises an analyte analogue which is a glucose derivative, a polymer or polysaccharide containing glucose or a carrier molecule covalently linked to a glucose derivative or glucose.

40. The use as claimed in claim 39 wherein said carrier molecule is a protein.

41. The use as claimed in claim 40 wherein said carrier protein is a serum albumin.

42. The use as claimed in any one of claims 27 to 41 wherein said protein forms part of a glucose biosensor.